in the adult. The cephalic ganglia give rise to the optic nerves; the rostral ganglia, which occupy a place on the supero-lateral face of the supra-œsophageal ganglia, are compared with the labial ganglia described by Tichomiroff in Bombyx, while the mandibular ganglia, which also enter into the composition of the brain, give rise to the sympathetic nerve.

The speculations which conclude the article, as to the homologies of the nervous system in various Metazoa, are not equal to the rest of the paper.—*J. S. K.*

MICROSCOPY.[1]

Method of Staining and Fixing the Elements of Blood.[2]—Recent discoveries of morphological elements in the blood hitherto unknown, as well as the newly published facts concerning its coagulation, have aroused an interest in the subject which calls for an acquaintance with the methods with which it is possible to follow those results. Accordingly, I would like to describe the method employed in this laboratory; for, although it has been mentioned by Professor Gaule in his lectures for several years, it has not as yet been published.

The methods formerly used were that of examining fresh blood and that, perfected by Ehrlich, which consisted in staining dried blood.

Our method consists in a series of manipulations requiring only thirty-five minutes for their completion.

The following is a list of the reagents, together with the length of time and the order in which each is to be used:

	Min.
1. Corrosive sublimate (concentrated solution)	6
2. Distilled water	1
3. Absolute alcohol	5
4. Distilled water	1
5. Hæmatoxylin (½ per cent. alum solution to which, for every 100 c.cm. employed, 20 drops 5 per cent. alcoholic solution have been added)	6
6. Distilled water	1
7. Nigrosin (½ per cent. water solution)	1
8. Distilled water	½
9. Eosin (1 gr. eosin dissolved in 60 c.cm. alcohol; 140 c.cm. distilled water)	2
10. Alcohol	5
11. Oil of cloves	1–2
12. Xylol.	
13. Canada balsam (diluted with xylol until it readily flows).	

As receptacles for these fluids, each person has upon his table

[1] Edited by C. O. WHITMAN, Milwaukee, Wisconsin.

[2] From the Physiological Laboratory at Zurich.

three shallow glass dishes with flat bottoms, so large that a slide may be easily put in and taken out of them. Into the first of these we pour corrosive sublimate, into the second distilled water, and into the third absolute alcohol. It is necessary either to label the dishes or to place the two not at the moment in use at one side. For the coloring fluids we use bottles whose stoppers serve at the same time as droppers or pipettes. The most convenient form has a glass stopper, which is hollow and drawn out into a fine point below, while above it broadens into a funnel with a lip whose opening is closed by a rubber membrane. A slight pressure upon the membrane causes, upon the removal of the finger, a rise of fluid in the funnel, which, upon the removal of the stopper from the bottle, can be at pleasure dropped upon the slide. For oil of cloves, xylol, and Canada balsam wide-mouthed bottles are used. In the first two bottles are brushes; in the last, the ordinary glass rod. Other necessary utensils are a glass rod, sharp-pointed scissors, clean slides and cover-slips, filter-paper, twine or coarse thread, a small bottle of absolute alcohol, a sharp, clean needle, a fine clean rag, and a hand-towel.

Aside from these, a board, fifteen by five inches, with two pair of holes, large enough for a piece of tape to pass through double, is an essential help. The first pair of holes should be four inches distant from the second, and the two holes of each pair one and a half inches apart. The tape should be so passed through the holes that there will remain upon one side of the board loops, on the other long ends, by which, upon passing the extremities of the frog through the loops, one may easily and firmly tie the frog upon the board. Such preparation is necessary, otherwise the manipulations cannot follow one another quickly enough. After these preliminaries have been completed, the labelled bottles being placed within reaching distance, the distilled water and alcohol in front of these, and the corrosive sublimate nearest of all, we are ready to bind our frog upon the above-mentioned board and begin our preparation. We make use of the frog for this purpose at first, since its blood coagulates less quickly than that of mammals. The vena femoralis, which may be seen as a dark blue line below the knee-joint on the inner side of the leg, having been snipped, we quickly bring with a glass rod a drop of the blood which comes from the wound upon a slide previously moistened by the breath, and throw the whole into the dish of sublimate for six minutes. If a little care is taken to spread out the drop of blood in putting it on the slide, the result is more satisfactory. Brought from the sublimate into the dish of water, we find that the greater part of the blood adheres to the slide. The superfluous sublimate being washed from the preparation during the moment that it remains in the water, we next partially dry the slide by resting it upon filter-paper before dropping it into the alcohol bath. The slide, which has remained in alcohol

six minutes, is brought again into distilled water for half a minute, since our coloring fluids are water solutions. The hæmatoxylin is then dropped upon the slide, and removed again at the end of six minutes by resting the edge of the slide upon filter-paper, and afterwards washing with distilled water for one minute. The same process follows with the nigrosin and eosin, the first remaining upon the slide for one minute, the second two minutes. From the eosin we bring the preparation directly into alcohol, since the eosin is partially an alcohol solution. At the end of five minutes the slide is taken out of the alcohol, and, in order to be quite sure that there is no water still clinging to the preparation, we incline the slide at a slight angle to the rag with which we are holding it, and pour a few drops of alcohol from the small bottle over it. If upon dropping oil of cloves on the preparation it should be dark upon a dark sleeve or other dark background, we may remove the oil of cloves with a few drops of xylol. Having quickly cleaned the slide close up to the preparation, we place a drop of Canada balsam upon it, which must be allowed to spread out before the cover-slip is lowered upon it.

Human blood is prepared in the same way, except that here the finger-tip undergoes the surgical operation. If a finger of the left hand be lightly bound with a string and a sharp needle be held in the right a quarter of an inch from the end, one quick energetic stroke suffices to bring a drop of blood to the surface, which should be transferred to the slide by drawing it, previously moistened, across the drop of blood.

A look at our preparations with the microscope shows us that the coloring substances we have used have attached themselves to certain parts and certain forms of corpuscles. In the preparation of the frog's blood we find that the large oval red corpuscles have been colored red with eosin. The nuclei are for the most part blue from hæmatoxylin, the well-known coloring substance for nuclei. The protoplasma, provided no coagulation has occurred, is homogeneous. The usually oval nuclei are also generally homogeneous, though occasionally granulated like the nuclei of other cells.

The white blood-corpuscles differ among themselves in form, color, and the number and size of their nuclei. 1. Those coarsely granulated which are deeply colored with eosin, hence their name "eosinophilous cells,"[1] are perhaps the most striking. Their form is usually round, and they contain from one to four nuclei. 2. A second kind is perhaps best characterized by its large nucleus sparely surrounded with protoplasma, colored blue with nigrosin. The form of the cell, according to the position in which we see it, is spindle-shaped, with an oval nucleus in which the granules

[1] This name was given by Ehrlich.

are distinct, and seem to be arranged in lines parallel to the long axis of the nucleus, or it is quite round with a round nucleus. The name "hæmatoblasts" was given them by Hayem. 3. Another variety has, like the "eosinophilous cells," several nuclei. Its protoplasma is, however, blue like that of the "hæmatoblasts," its form irregular, recalling the forms that the amœba is wont to assume; accordingly such cells have been called "amœbocytes." 4. Occasionally one sees still another cell, whose single large nucleus is oval or irregular in outline and lies in protoplasma like that of the "amœbocyte." These cells are larger than the other white blood-corpuscles, and contain here and there foreign bodies, such as pigment-granules and drops of fat in their protoplasma. They are called on account of their form "endotheloid cells." With further study of the preparation other forms are found, which may be looked upon as intermediate between "hæmatoblasts" and "amœbocytes," for in some cases the corpuscles have nuclei like "hæmatoblasts," whereas the protoplasm has increased in amount and sent out projections like the pseudopodia of an amœba; in others the nucleus is round instead of oval; in others still the nucleus seems to be in the act of falling into two parts.

These latter forms suggest the idea that a relation may exist between "amœbocytes" and "hæmatoblasts," but what the relation may be, whether the change is from "amœbocyte" to "hæmatoblast" or the opposite, whether the "eosinophilous cells" and "endotheloid cells" are in any way related to them and to one another, cannot be determined by the method just described. Two courses lie open to us in our attempt to answer these questions: 1, to examine the same blood at intervals after it has been taken from the frog; or, 2, to watch changes in fresh blood which has been protected from evaporation. To do the first, we have simply to place a slide with a drop of blood upon it in a moist chamber,[1] and after certain intervals (five minutes, fifteen minutes, half an hour, two hours) to fix and color the blood as above. If we examine a preparation fixed at the end of two hours, the whole aspect is changed. We find representatives of the different forms, but not in the same proportion. The "endotheloid cells" have become more numerous and the other forms less so. The former have also become much larger, with broad hyaline borders. The granules of the protoplasma are coarser about the nucleus, but constantly smaller and less distinct towards the hyaline border. Between the protoplasma-granules are frequently pigment-crystals and bodies colored with eosin. These foreign bodies lie often in clear oval spaces next to the nucleus; otherwise these spaces are empty, or

[1] The moist chamber is easily constructed by covering the bottom of a flat-bottomed dish with wet filter-paper and placing a ground-edged cover upon the dish, whose edges should also be ground.

contain a small nucleus, a clump of yellow pigment, or a body closely resembling a small red blood-corpuscle. To control this experiment we may make use of another one,—that is, we may cover a fresh drop of blood with a cover-slip and seal it from the air.[1] Thus the blood coagulates slowly, and we may study directly the changes the forms undergo during coagulation. The granules of the "eosinophilous cells" may be seen to become larger, less distinct, and disappear. The "eosinophilous cell" has developed into the "amœbocyte." The "hæmatoblasts" assume the forms mentioned above, the nucleus and cell as a whole become round, and at length send out pseudopodia in every direction, so that it is impossible to distinguish them from "amœbocytes." The "amœbocytes," in their turn, at first stretch out their pseudopodia in a lively manner, then gradually attach themselves to the cover-slip, where they spread themselves over a large surface, and resemble the "endotheloid cells" with their broad borders of hyaline substance and the granulated protoplasma about the nucleus. If we now bring together the facts we have observed,—1, in instantly fixed blood; 2, in blood fixed after intervals; 3, in fresh blood,—we find that the first three kinds of white blood-corpuscles may at length become "endotheloid cells."

What is, then, the fate of the "endotheloid cells"? Are the bodies we have described as lying in their protoplasma and resembling incomplete blood-corpuscles to be considered as such? The endothelial cells which they resemble are, as is known, broad, flat cells that lie spread out on the inner surface of the blood-vessels similarly as the "endotheloid cells" flatten themselves out on the cover-slip. Their protoplasma is colored with nigrosin, and in the small capillaries, where one or two cells suffice to form the circumference of the capillary, has been observed to contain pigment and more or less developed red blood-corpuscles. Especially is this the case in the liver and spleen of the frog. If the spleen be teased out, and its cells fixed and colored in the manner mentioned above, not only do we find that the number of white blood-corpuscles, especially of the "endotheloid cells," is much larger in proportion to the red blood-corpuscles than it is in circulating blood, but other cells are present which possess the general characteristics of "endotheloid cells" and endothelial cells. They are richer in pigment, contain often several undeveloped red corpuscles, and cling together in groups. Gaule, in his Strassburg lecture, called these cells "ammenzellen," because in them he observed the development of the red blood-corpuscles. In the course of his observations of a series of frogs he noticed that the "ammenzel-

[1] The edges of the cover-slip must be thoroughly free from moisture, a bit of melted wax dropped upon every corner, and the wax then drawn along the edges of the cover-slip with a heated iron wire.

len" which lie in groups similar to the follicles of the animal spleen, between the arteries entering and the veins leaving the spleen on the periphery, undergo significant changes, normally, in the course of the winter, under the influence of pilocarpine, in a few hours. The result in both cases was the same. The "ammenzellen," at first rich in pigment, lose their pigment as the number of undeveloped corpuscles increases. At the same time the number of corpuscles in the circulating blood was counted, the result showing that as the pigment of the "ammenzellen" decreased the number of the circulating red corpuscles became greater, the quantity of undeveloped corpuscles increased, and that many of the circulating corpuscles were still bordered with granules of pigment. Another indication that blood-building elements are present in the "ammenzellen" was the iron reaction which the protoplasma gave with potassium ferrocyanide. From these observations it seems hardly to be doubted that red blood-corpuscles are developed in the "ammenzellen," and partially at least in the endothelial cells, and in the "endotheloid cells." The relation in which these three cells stand to the blood-vessels remains to be considered. The blood-vessels of the embryo have their origin, as the embryologists have taught us, in the mesoderm in chains of endothelial cells which contain clear spaces in their protoplasma that later communicate with one another to form a fine capillary, in whose walls the first red blood-corpuscles are formed. Returning to the spleen, we recall the fact that the "ammenzellen" groups lie between the capillaries of the arteries, with their endothelial cells on the one hand and the capillaries of the veins on the other hand, and that between the in-flowing and out-flowing vessels the regular blood-vessels with their lining cells fail. It is, then, not difficult to suppose that the "ammenzellen" and the "endotheloid cells," which are so numerous in the spleen, might be the stage upon which, as in the mesoderm of the embryo, a constant building of new blood-vessels and blood-corpuscles is taking place. The white blood-corpuscles of the frog may perhaps be looked upon as undeveloped "ammenzellen," though their origin and the functions peculiar to each form are not yet clear. It is significant that a seeming relation exists between the coagulation of the blood and the formation of white blood-corpuscles, for as the blood of the frog begins to coagulate the "hæmatoblasts" become especially numerous and group themselves characteristically; but to this point we shall refer again in connection with human blood, which is in many points similar to the blood of the frog.

The red blood-corpuscles of human blood contain, as is known, no nuclei. In our preparation they retain the disk form and color, like the protoplasma of the red corpuscles of the frog with eosin. The white blood-corpuscles are represented by the two forms "eosinophilous cells" and "amœbocytes." The "hæmatoblasts,"

as such, are wanting in human blood, but since we have had our attention directed by Hayem to the fact that the "hæmatoblasts" play an important part in the coagulation of the frog's blood, it is possible to think that some element is present in mammalian blood which also acts as a factor in coagulation. The coagulation of the frog's blood begins with the grouping of the "hæmatoblasts" into a rosette form. The red corpuscles then arrange themselves radially about this point as a centre. Do we find an analogous process at the commencement of the coagulation of mammalian blood? The blood of mammals coagulates very rapidly, whereas that of the frog changes very slowly; hence, if we would study the blood of mammals before coagulation, we must prevent this process by means of some reagent. Such an experiment cannot be tried with a human being, but is easily made with a dog. The reagent usually employed is peptone, which is injected in solution into the jugular vein of the dog, the amount injected being 0.3 grain peptone for every kilogramme weight of the dog. The microscopical examination of blood in which coagulation has thus been prevented shows that there exist in the blood, aside from the other elements, tiny tablet-like granules which tend to cling together in clumps. These elements were described by Bizozerro, and called by him "blutplättchen." It thus seems probable that the "blutplättchen" have something to do with the coagulation of the blood. That they also exist in human blood is evident from their presence in our preparation as small, faintly-tinged bodies, which lie in groups of twos and threes together. They did not disappear from the blood we employed, because we did not give it time to coagulate before fixing it. Therein lies the advantage of this method in the examination of human blood. It gives us not only the possibility to distinguish the different elements of the blood, but through it, it has been possible to discover elements which, like the "hæmatoblasts," accompany the phenomenon of coagulation, and also to determine in part the relation that exists between the elements. It would not agree with the general plan of nature if every form did not play a different rôle in the organism, and after all that has been discovered it is not improbable that we shall one day be able, through watching the changes which the different elements undergo in the blood, to discover the disturbances caused by different ferments and organisms in the blood. Thus we think that the hope of clever physicians may one day be verified, that the analysis of a drop of blood may give a clue to the pathological changes in the body.—*Alice Leonard Gaule.*

PSYCHOLOGY.

Intelligence of Echinoderms.—The experiments of Professor Preyer upon starfish and ophiurids tend to prove that they are